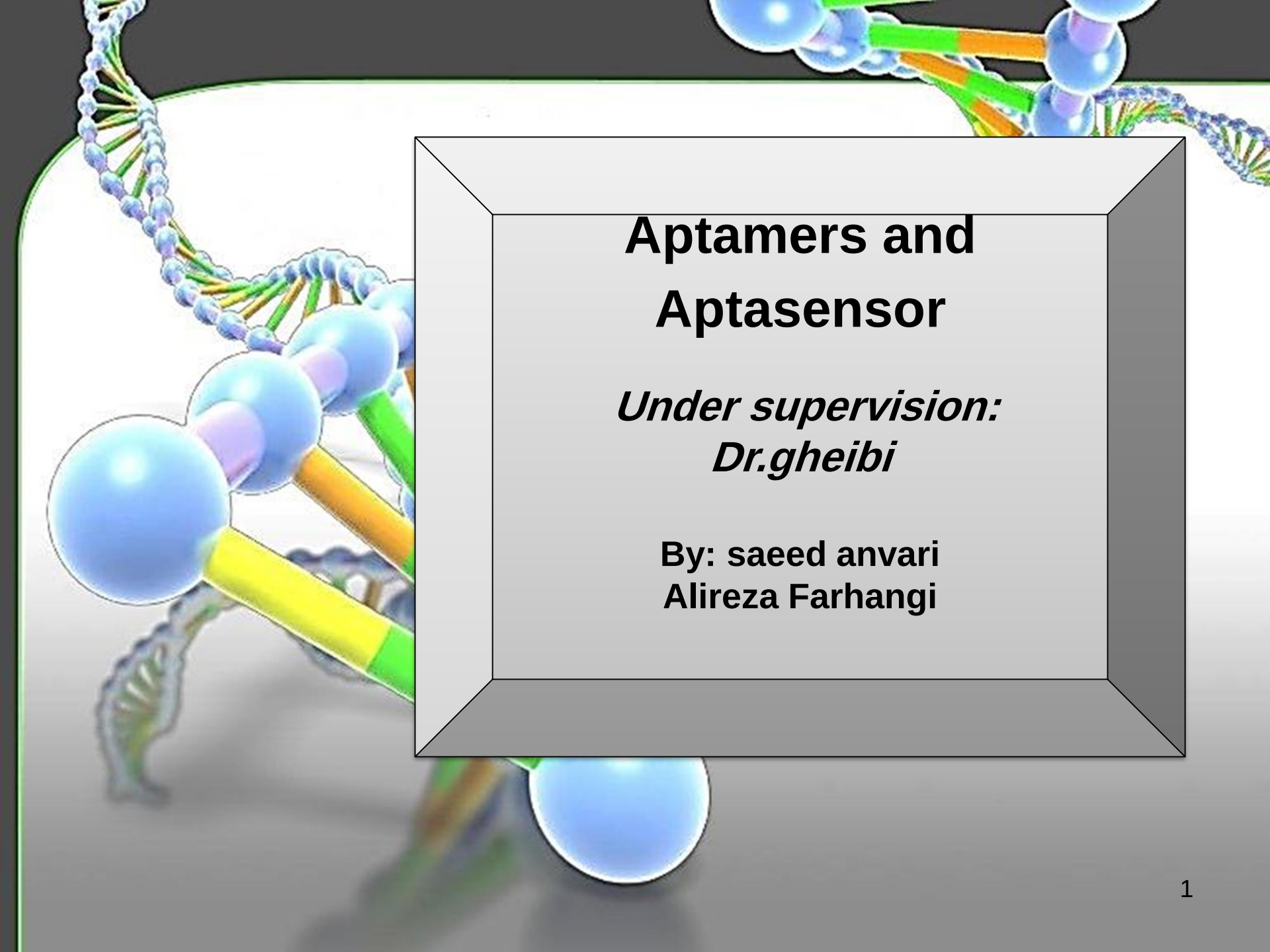


Aptamers and Aptasensor

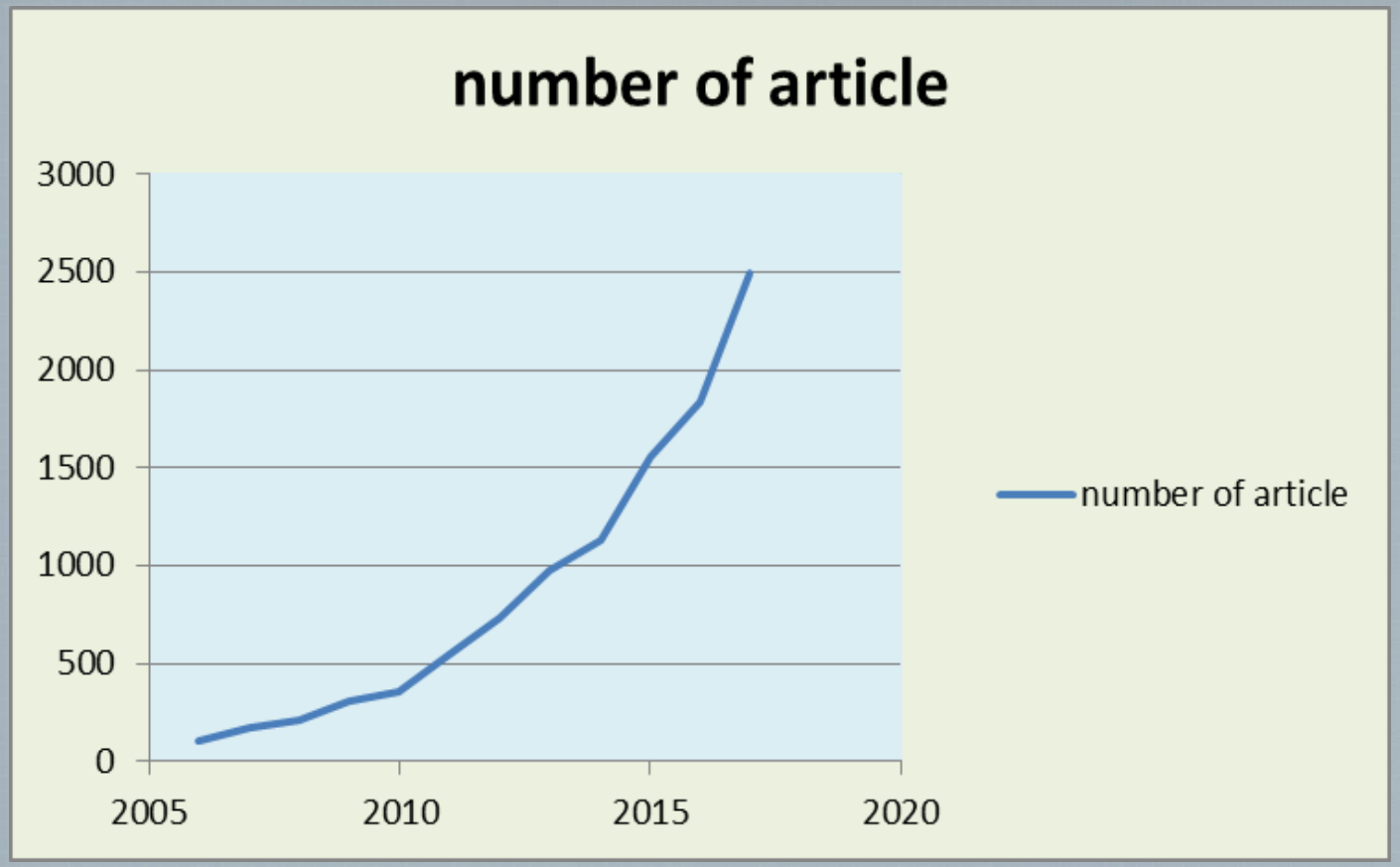
Under supervision:
Dr.gheibi

By: saeed anvari
Alireza Farhangi

Content

- Introduction
- Discussion
- Conclusion
- Reference

Research trend



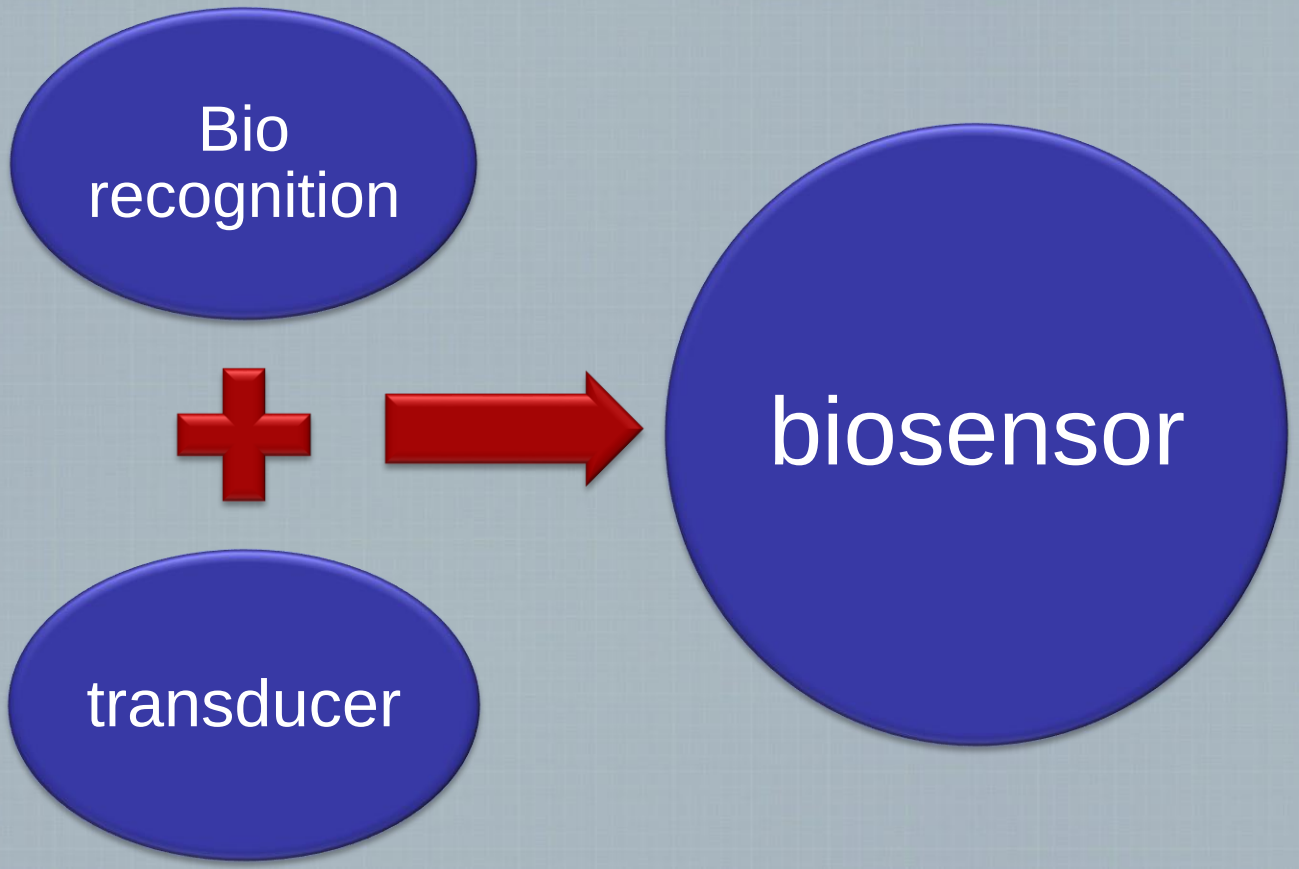
https://scholar.google.com/ last seen:2018/14/01

Introduction

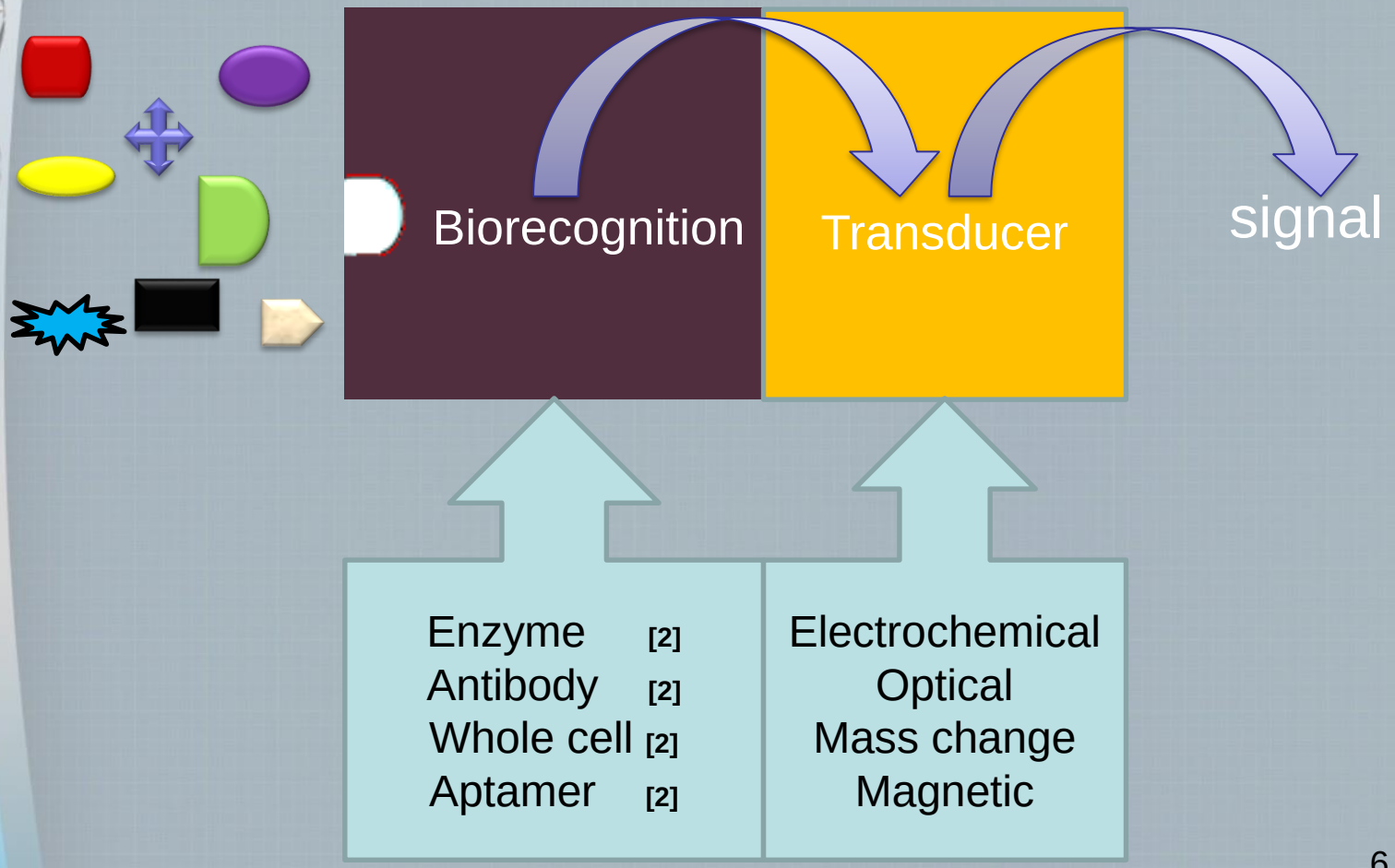
Introduction

biosensor

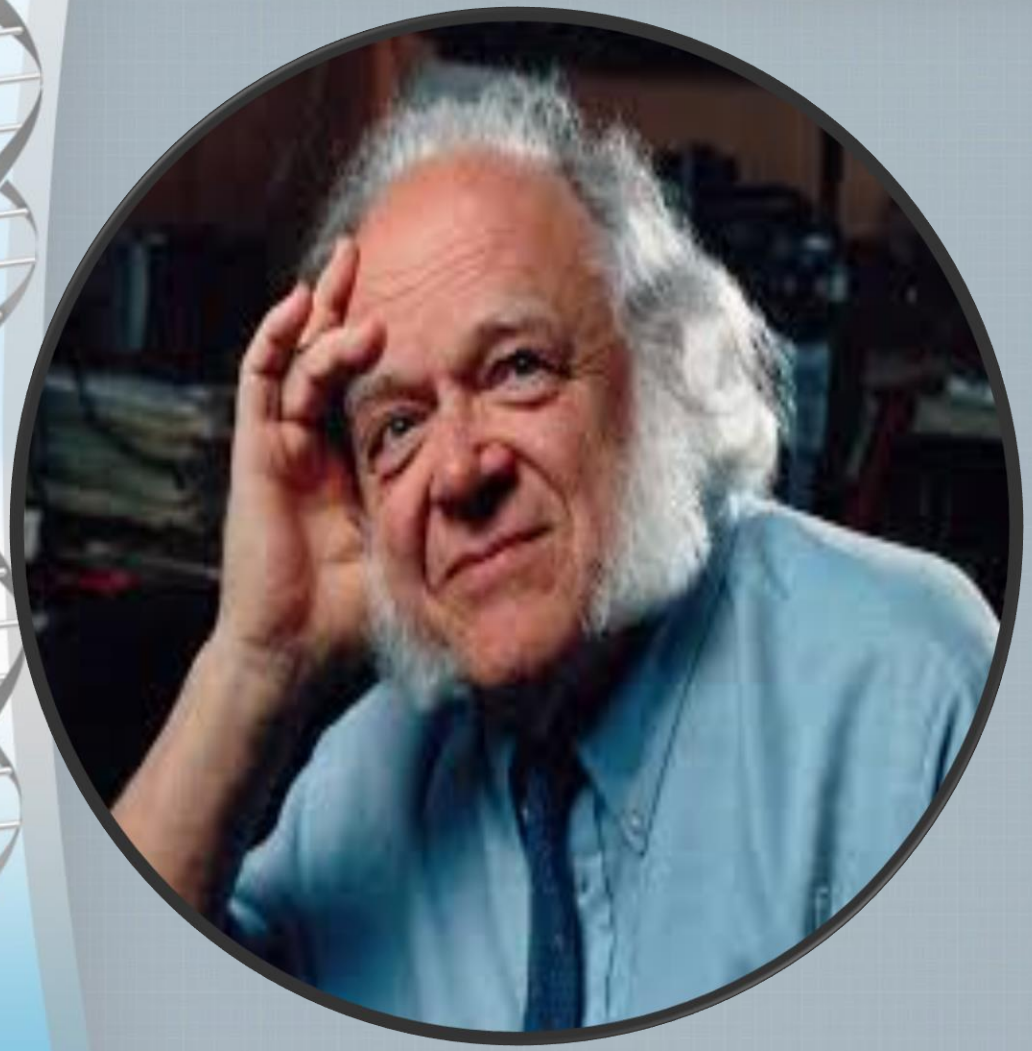
- Biosensors are devices detecting the presence of a target [1]



Biosensor



Introduction



professor leland clark

father of biosensor

blood glucose

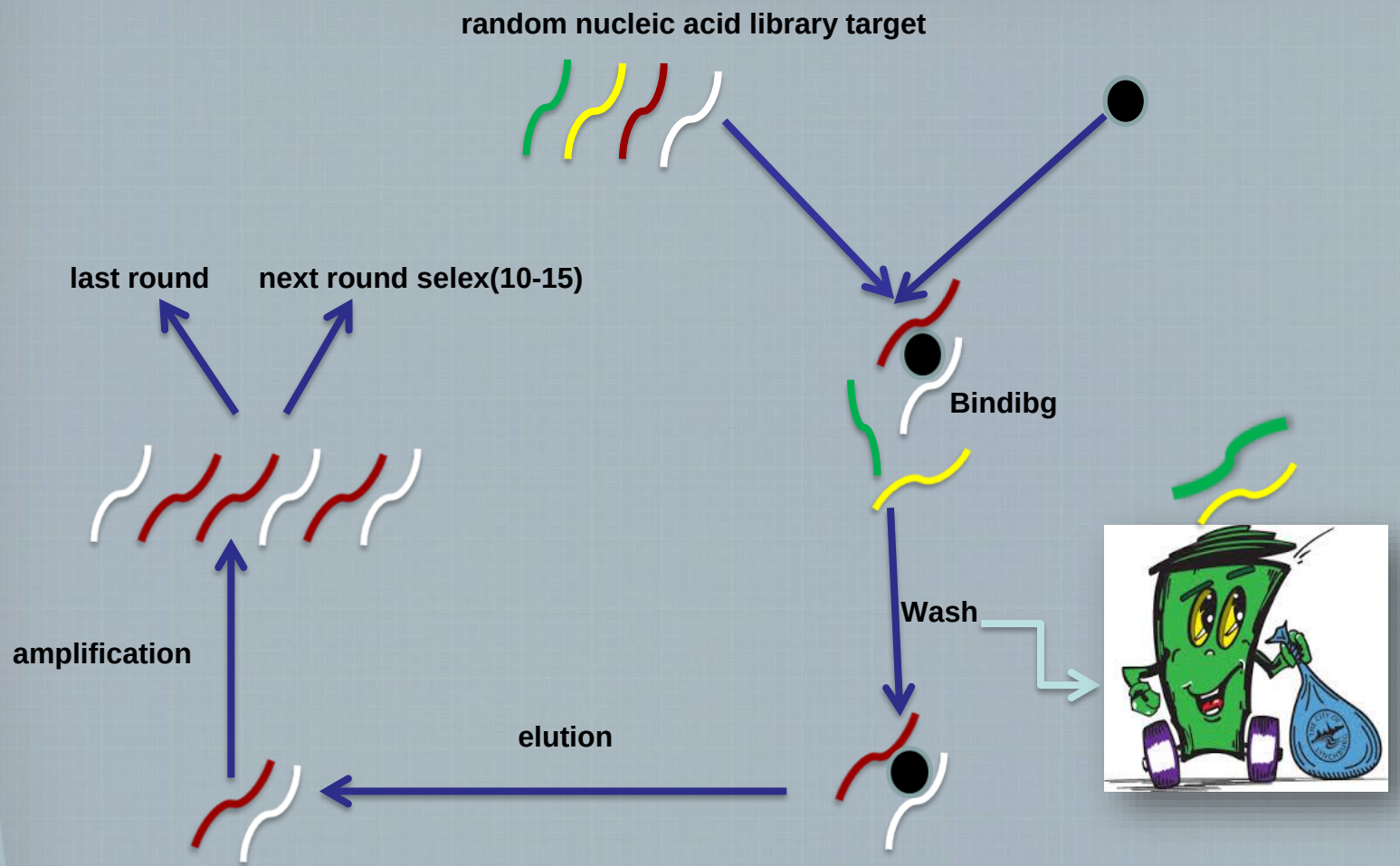
(1918-2005)

Introduction

aptamer

- oligonucleotides that specifically bind various target
- usually from 10^{14} to 10^{15} nucleotides [3]
- Aptamer target : proteins, peptides, amino acids, drugs, metal ions and even whole cells [3]
- isolated via SELEX(Systematic Evolution of Ligands by Exponential Enrichment)

Selex [4]



Introduction

selex

Year	SELEX type	References *
1990–1993	Classic, Negative	1,2,5
1994	Counter or Subtractive	6,7
1995	Blended (Covalent), Photoselex (crosslinked), cDNA-SELEX	8–10
1996	Spiegelmer isolation	12
1997	*In vivo*	13
1998	Chimeric	14
1999	Multistage Cell Specific SELEX(CS-SELEX)	15
2000	Beacon aptamers, Indirect	16–18
2001	Toggle	19,20
2002	Expression cassette	21
2003	Tailored-SELEX	22
2004	CE-SELEX	23
2005	FluMAG	24
2006	TECS-SELEX, NON-SELEX (NCEEM)	25,27
2007	NanoSelection® (nM-AFM SELEX), MonoLEX	28,30
2008	CS-SELEX	31,32
2009	Next-generation SELEX	33
2010	Microfluidic-SELEX, Bioinformatics analyses	36,37,43,44
2011	Multiple-target high-throughput SELEX	38–41

http://aptamerstbc2013.wixsite.com/aptamers/history

Review

SELEX experiments: New prospects, applications and data analysis in inferring regulatory pathways

Marko Djordjevic *

Mathematical Biosciences Institute, The Ohio State University, Columbus, OH 43210, United States

Received 31 December 2006; received in revised form 2 March 2007; accepted 6 March 2007

Abstract

Systematic Evolution of Ligands by EXponential enrichment (SELEX) is an experimental procedure that allows extraction, from an initially random pool of oligonucleotides, of the oligomers with a desired binding affinity for a given molecular target. The procedure can be used to infer the strongest binders for a given DNA or RNA binding protein, and the highest affinity binding sequences isolated through SELEX can have numerous research, diagnostic and therapeutic applications. Recently, important new modifications of the SELEX protocol have been proposed. In particular, a modification of the standard SELEX procedure allows generating a dataset from which protein–DNA interaction parameters can be determined with unprecedented accuracy. Another variant of SELEX allows investigating interactions of a protein with nucleic-acid fragments derived from the entire genome of an organism. We review here different SELEX-based methods, with particular emphasis on the experimental design and on the applications aimed at inferring protein–DNA interactions. In addition to the experimental issues, we also review relevant methods of data analysis, as well as theoretical modeling of SELEX.

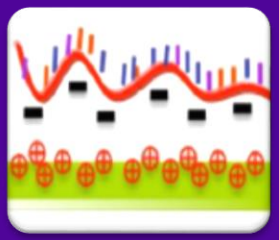
Keywords: *In vitro* selection; High-throughput SELEX; SELEX-SAGE; Genomic SELEX; SELEX modeling; Protein–nucleic acid interactions

Introduction

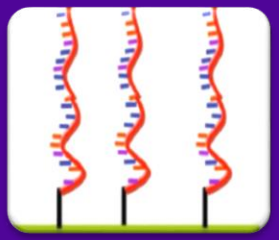
Immoblize

- physicochemical properties both surface and DNA

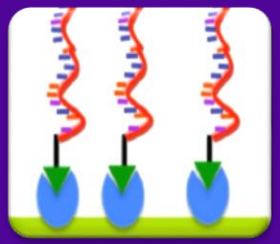
three important mechanisms [5]



physical adsorption



covalent



Streptavidin-biotin

Introduction

Physical adsorption

Simple

Any modification on dna

Ionic intraction: negative charge dna And positive charge covering surface

Amin, Nitrocellulose [5]

Advantage:

Fast

Direct method(no linker)

drawback:

Deabsorbtion by detergent

Deabrorbtion by change ph,ionic strength

Introduction

covalent

Necessary for molecule:

Do not adsorb

Adsorb very weakly

Require modification either oligonocleoutide ,surface

Advantage:

Good satablity [6]

high bindin strength

Drawback:

slow

Introduction

covalent

Dna prob modification

CHO(aldehyde)	amines(NH2)	[7]
N=C=S(Isothiocyanate)	amines(NH2)	[8]
Si-R-SH(Mercaptosilane)	Thiol(SH)	[9]
$HC_2(CO)_2NH$(Maleimide)	Thiol(SH)	[10]

* Aptamer immobilized on surface via 3’ end [11]
* Thiol sulfhydryl group direct bonding and a gold surface [12]

Introduction

Streptavidin(avidin)-Biotin

Streptavidin(avidin) have 4 binding site for biotin

Change buffer ph, high tempreture not affect on complex

Commoly use gold electrod

advantage:

Increase amount of aptamer on sensor surface [13]

Darwback:

Expensive

Multistep

Introduction

Biothin method

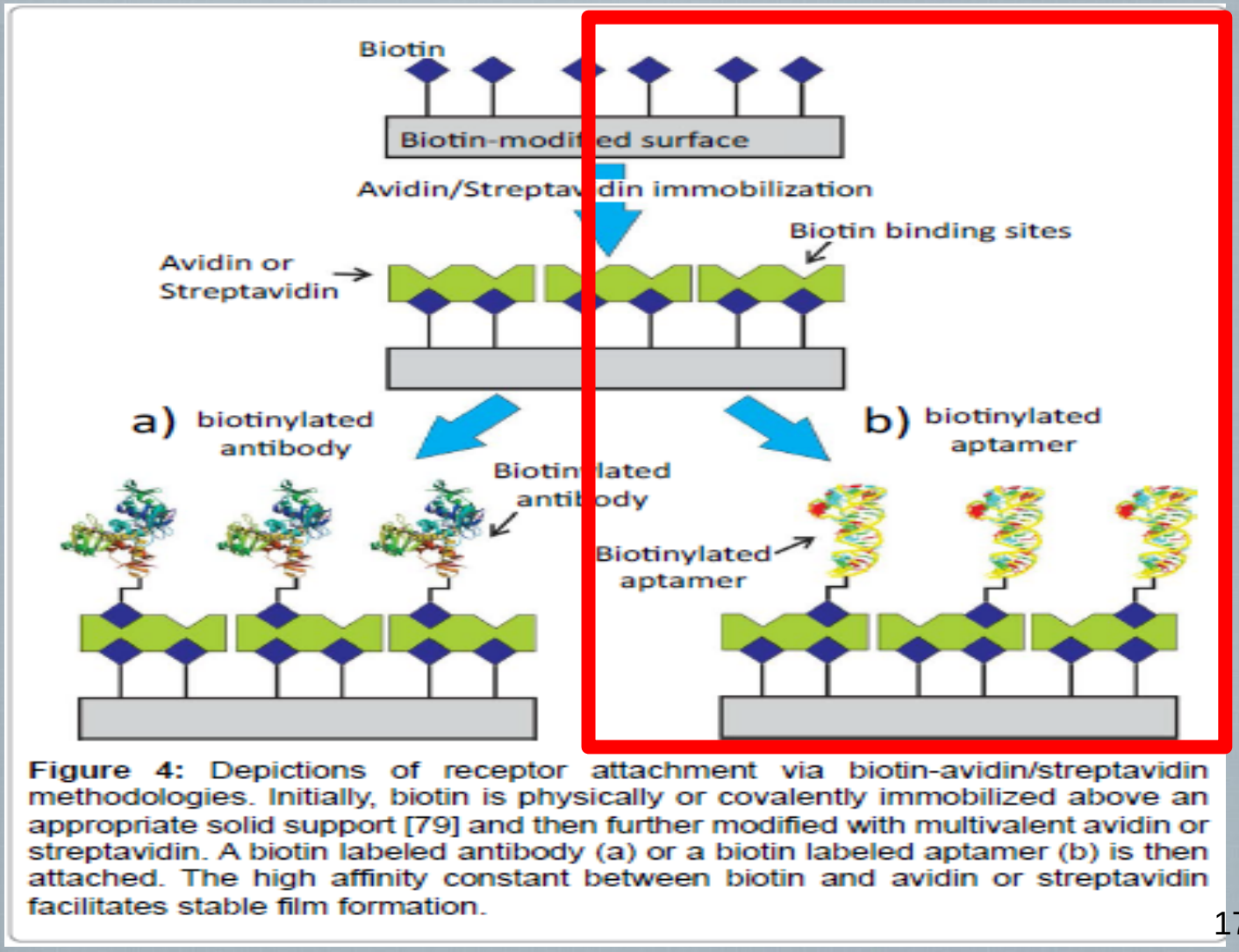
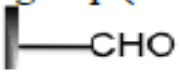
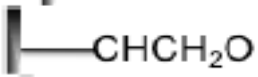
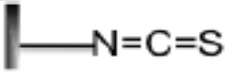
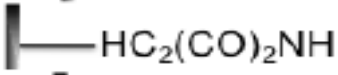
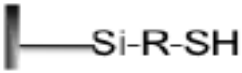


Figure 4: Depictions of receptor attachment via biotin-avidin/streptavidin methodologies. Initially, biotin is physically or covalently immobilized above an appropriate solid support [79] and then further modified with multivalent avidin or streptavidin. A biotin labeled antibody (a) or a biotin labeled aptamer (b) is then attached. The high affinity constant between biotin and avidin or streptavidin facilitates stable film formation.

Introduction

Surface Property	Group Structure	DNA Probe Modified	Immobilization Method
Amine		None	Physical absorption
Nitrocellulose		None	Physical absorption
Poly(l-lysine)		None	Physical absorption
PAAH		None	Physical absorption
Diazonium ion		Non	Physical absorption
Gold (Au)	Au surface	Thiols (-SH)	Chemisorption
Carboxyl (with EDC)	-COOH group (with EDC)	Amines ($-NH_2$)	Covalent
Aldehyde		Amines ($-NH_2$)	Covalent
Epoxy		Amines ($-NH_2$)	Covalent
Isothiocyanate		Amines ($-NH_2$)	Covalent
Maleimide		Thiols (-SH)	Covalent
Mercaptosilane		Thiols (-SH)	Covalent
Streptavidin		DNA-Biotin	Non-Covalent
Avidin		DNA-Biotin	Non-Covalent

Review

Immobilization Techniques for Microarray: Challenges and Applications

Satish Balasaheb Nimse [1], Keumsoo Song [2], Mukesh Digambar Sonawane [1], Danishmalik Rafiq Sayyed [1] and Taisun Kim [1,*]

Abstract: The highly programmable positioning of molecules (biomolecules, nanoparticles, nanobeads, nanocomposites materials) on surfaces has potential applications in the fields of biosensors, biomolecular electronics, and nanodevices. However, the conventional techniques including self-assembled monolayers fail to position the molecules on the nanometer scale to produce highly organized monolayers on the surface. The present article elaborates different techniques for the immobilization of the biomolecules on the surface to produce microarrays and their diagnostic applications. The advantages and the drawbacks of various methods are compared. This article also sheds light on the applications of the different technologies for the detection and discrimination of viral/bacterial genotypes and the detection of the biomarkers. A brief survey with 115 references covering the last 10 years on the biological applications of microarrays in various fields is also provided.

Keywords: microarray; 9G technology; biosensors; DNA self-assembly; hybridization; biomarker

Types of transducer and methods

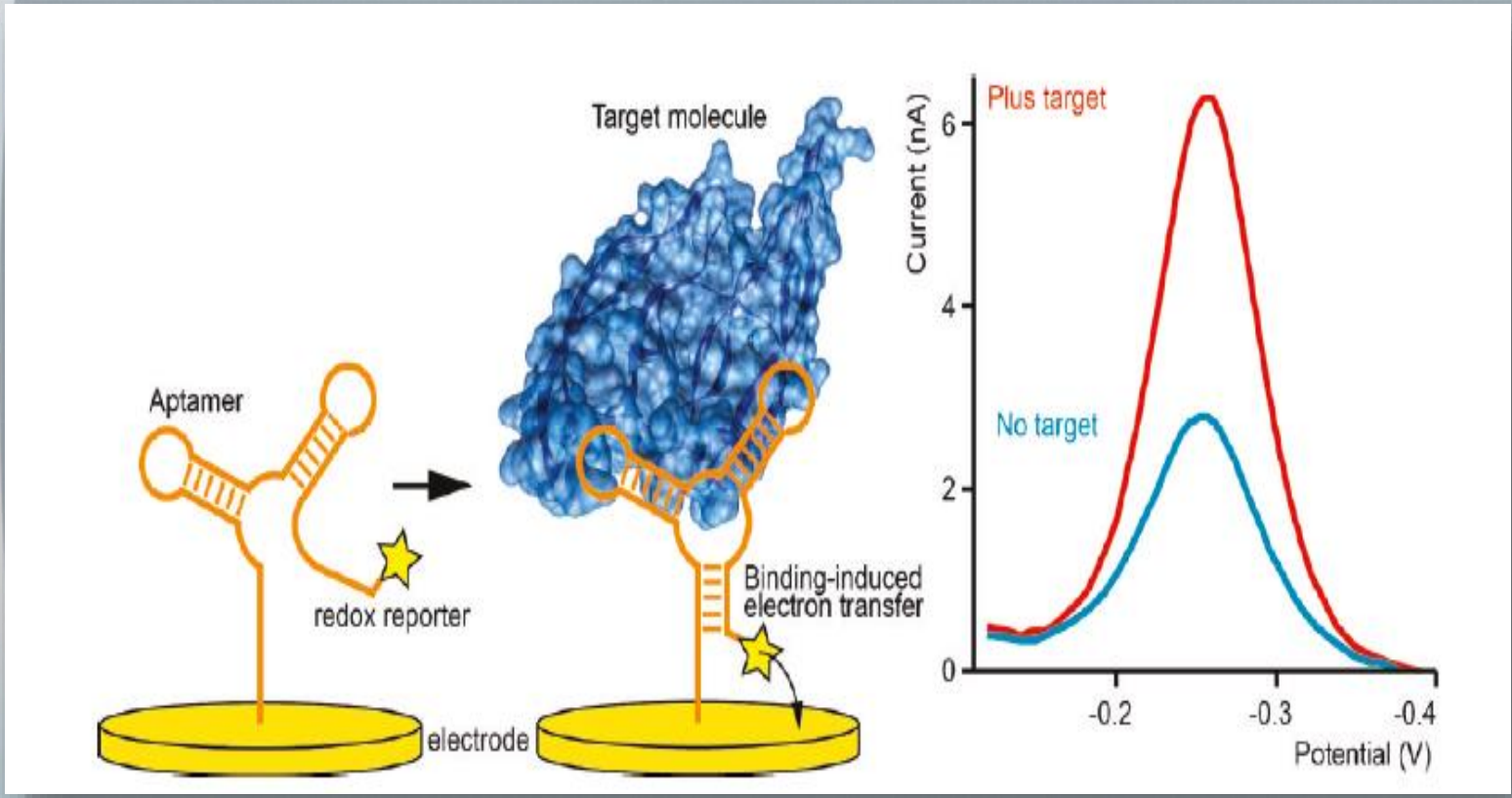
Types of transducer and methods

Electrochemical

TISS[14]
Target-induced structure switching mode

Types of transducer and methods

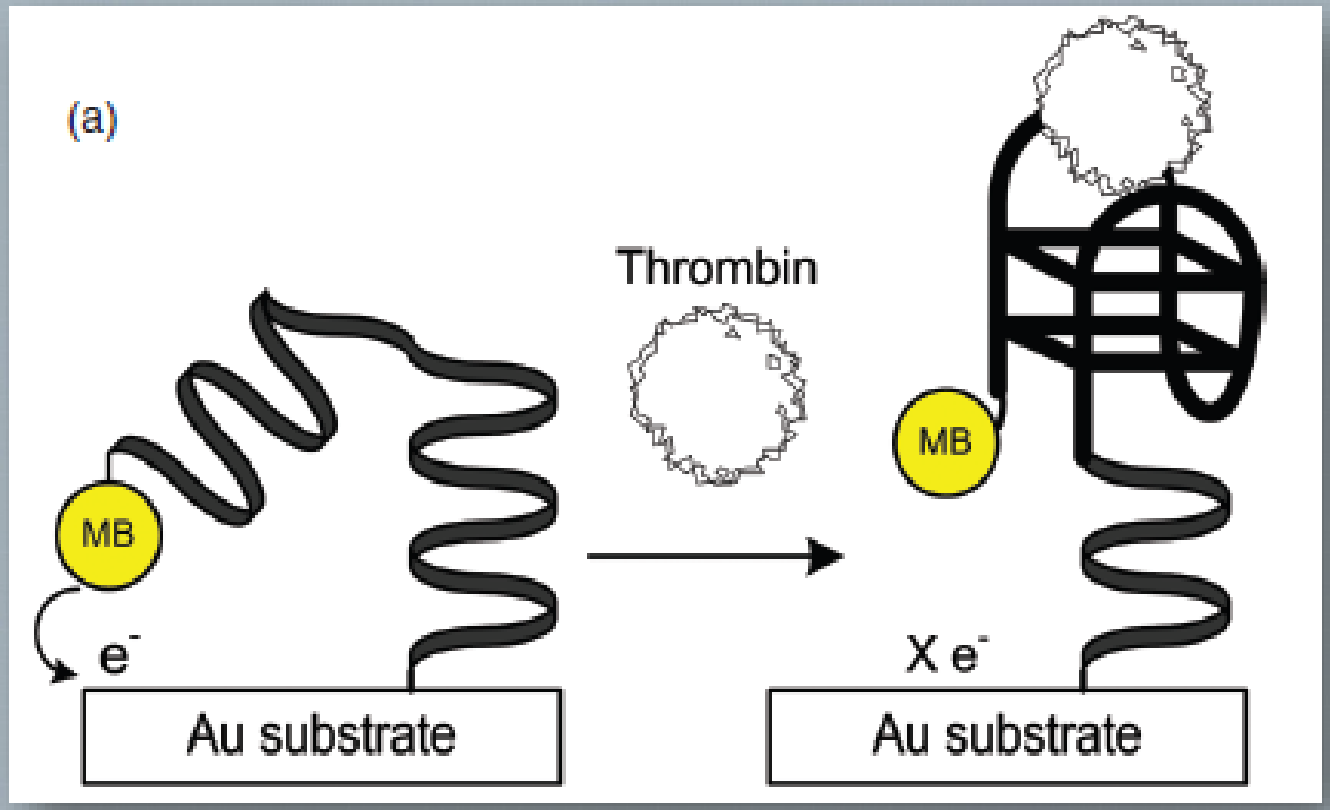
- **Electrochemical ⟶ TISS (signal ON)**



http://pubs.rsc.org/en/content/articlelanding/2013/an/c3an00818e/unauth#!divAbstract

Types of transducer and methods

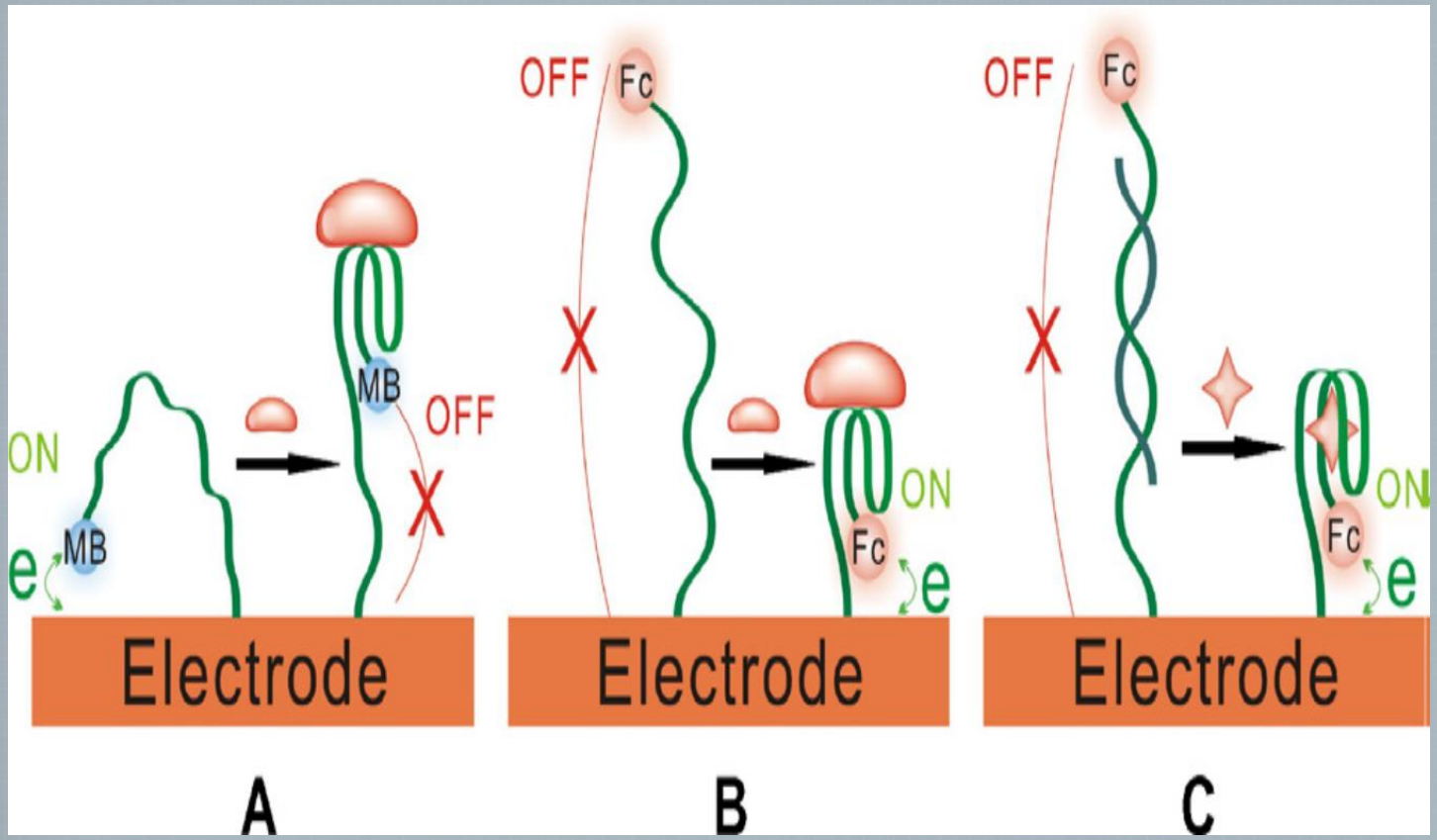
- **Electrochemical ⟶ TISS (signal off)**



http://pubs.rsc.org/en/content/articlelanding/2013/an/c3an00818e/unauth#!divAbstract

Types of transducer and methods

- **Electrochemical ⟶ TISS**



https://www.sciencedirect.com/science/article/pii/S0165993607002658

Review Article

Electrochemical Aptamer-Based Biosensors: Recent Advances and Perspectives

Department of Chemistry, Faculty of Science, Mansoura University, Dumyat 34517, Egypt

Correspondence should be addressed to Abd-Elgawad Radi, abdradi@yahoo.com

Received 17 February 2011; Revised 12 May 2011; Accepted 16 May 2011

Academic Editor: Farnoush Faridbod

This paper reviews the advancements of a wide range of electrochemical aptamer-based biosensors, electrochemical aptasensors, for target analytes monitoring. Methods for immobilizing aptamers onto an electrode surface are discussed. Aptasensors are presented according to their detection strategies. Many of these are simply electrochemical, aptamer-based equivalents of traditional immunochemical approaches, sandwich and competition assays employing electroactive signaling moieties. Others, exploiting the unusual physical properties of aptamers, are signal-on (positive readout signal) and signal-off (negative readout signal) aptasensors based on target binding-induced conformational change of aptamers. Aptamer label-free devices are also discussed.

Types of transducer and methods

- **Optical Aptasensor (colorimetric)** [15]

  For the detection of small molecules

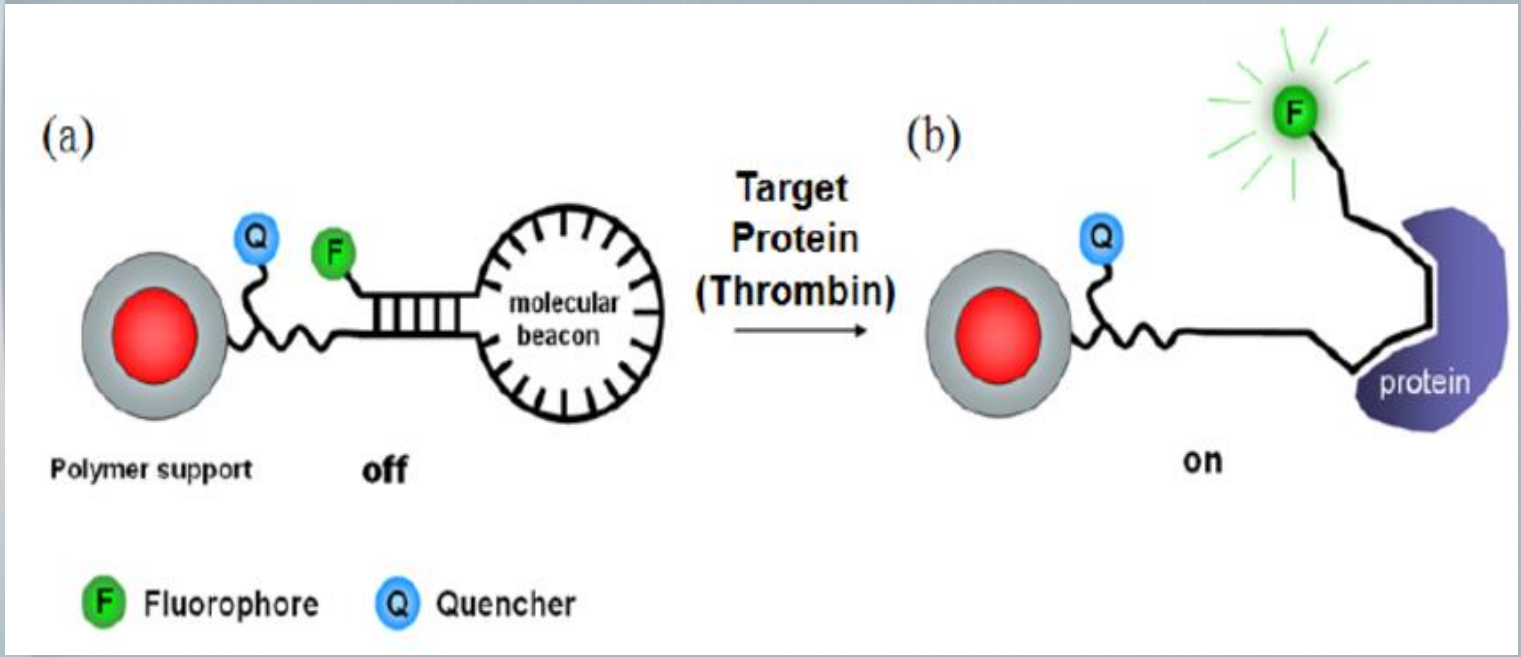
  ATP,adenosine,cocaine,dopamine,kanamycin,glucose

- **fluorescence**
- **Nano particles**
- **Nano materials**

Types of transducer and methods

- **Optical Aptasensor**

  fluorescence [16]

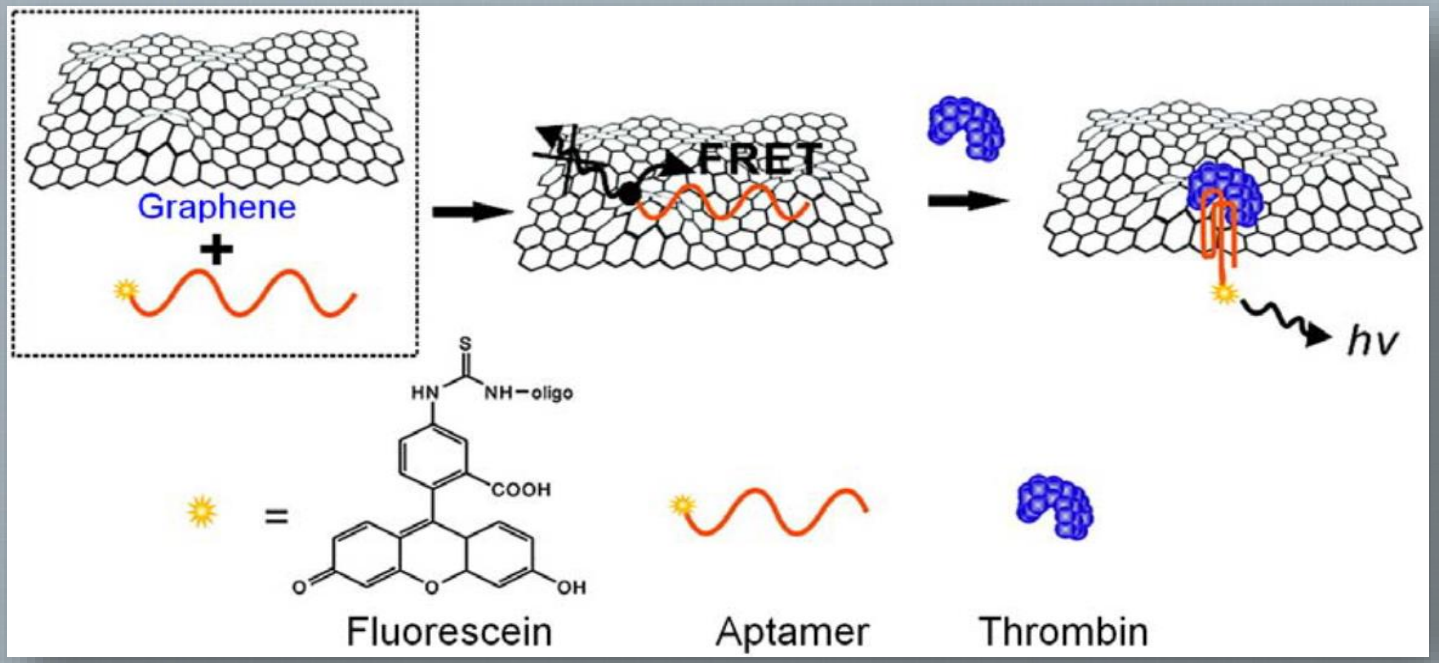


https://www.researchgate.net/figure/221881231_Figure-4-Schematic-illustration-of-MBs-on-optically-encoded-beads-for-detect

Types of transducer and methods

- **Optical Aptasensor**

Fluorescence [17]

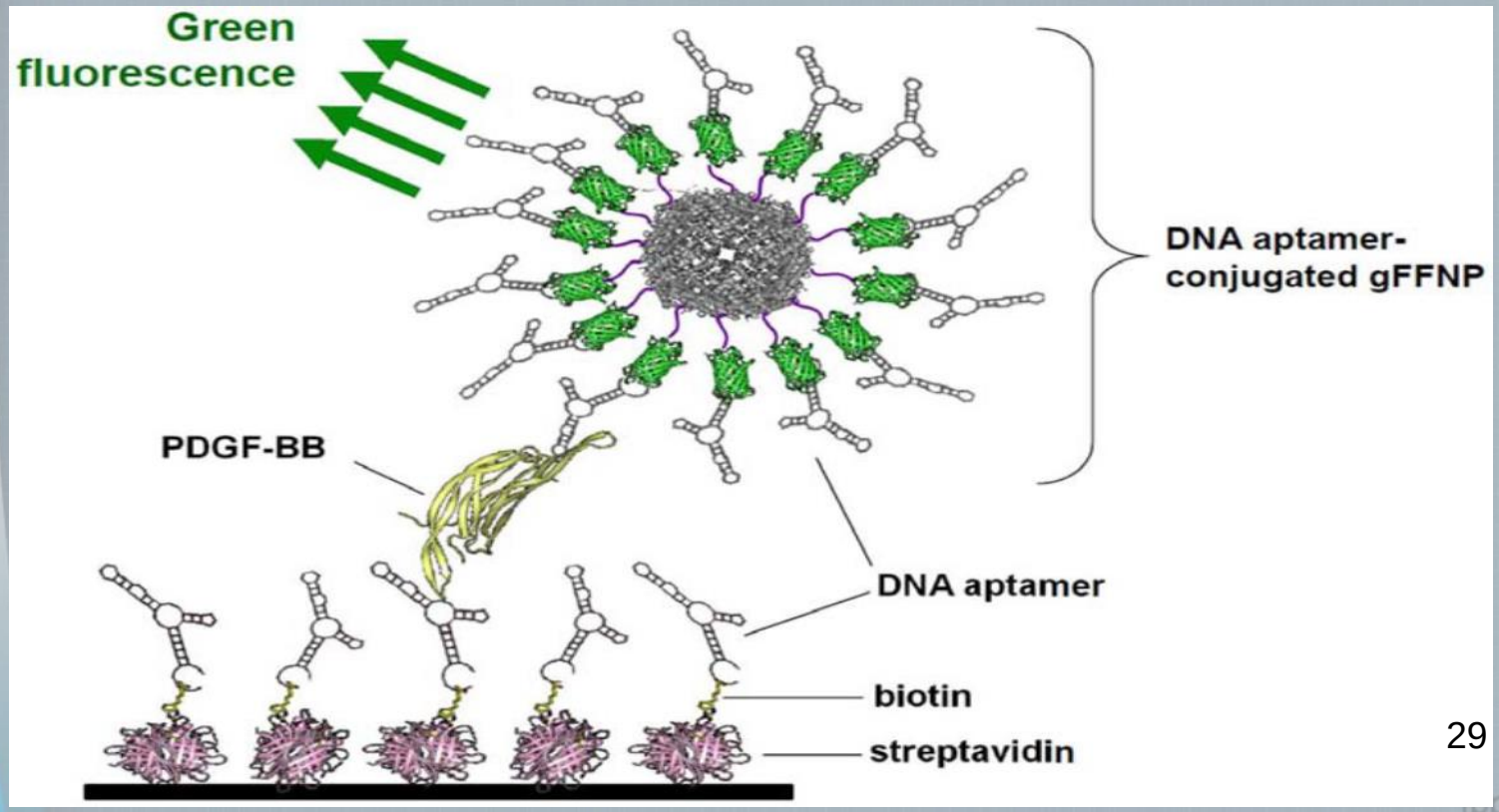


Target-induced structure switching mode

Types of transducer and methods

- **Optical Aptasensor ⟶ Fluorescence**

Sandwich Mode [18]



Types of transducer and methods

- **Optical Aptasensor**

Nano particle

Conjugation of aptamers on various nanoparticles has led to highly sensitive and selective Aptasensors.

Properties of nano particles [19]

1)size/shape-dependent optical properties

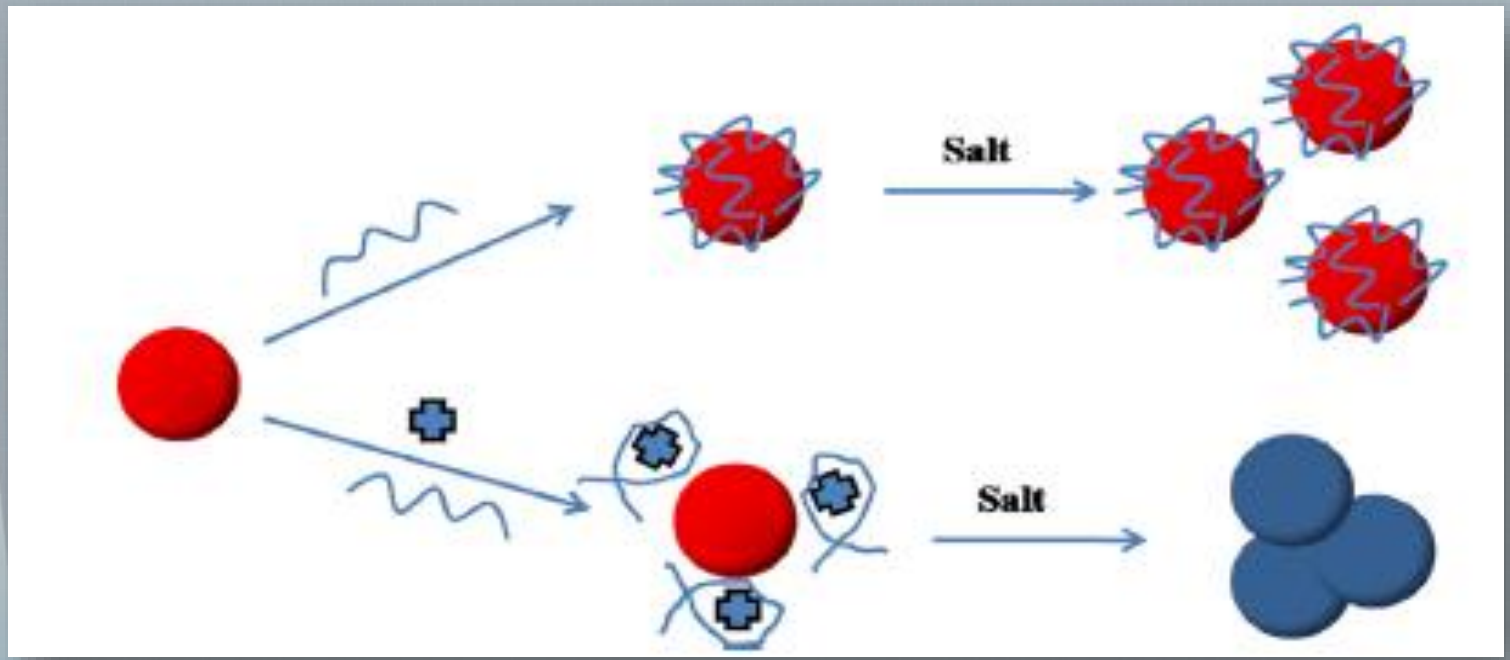
2)easy tuning of surface properties

3)catalytic ability

Types of transducer and methods

- **Optical Aptasensor**

Nano particle (lable free) [20]

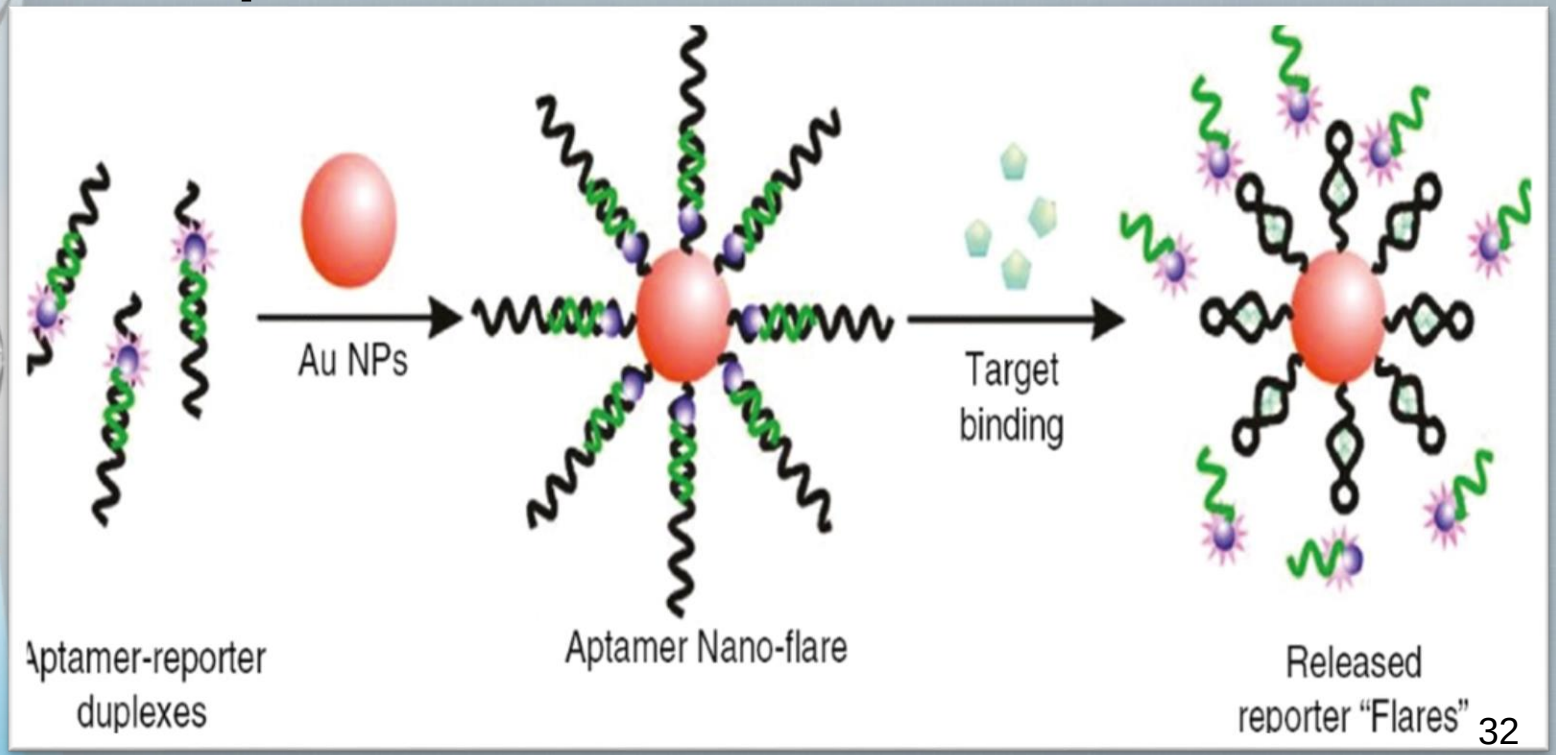


http://www.sciencedirect.com/science/article/pii/S095656631400195X

Types of transducer and methods

- **Optical Aptasensor**

Nano particle and Fluorescence [21]



Introduction

Types of transducer and methods

- **Optical Aptasensor ⟶ Nano materials**

Carbon nanomaterial

single walled carbon nanotubes (SWCNTs)

- Properties carbon nanotubes [22]

1)Higher surface area

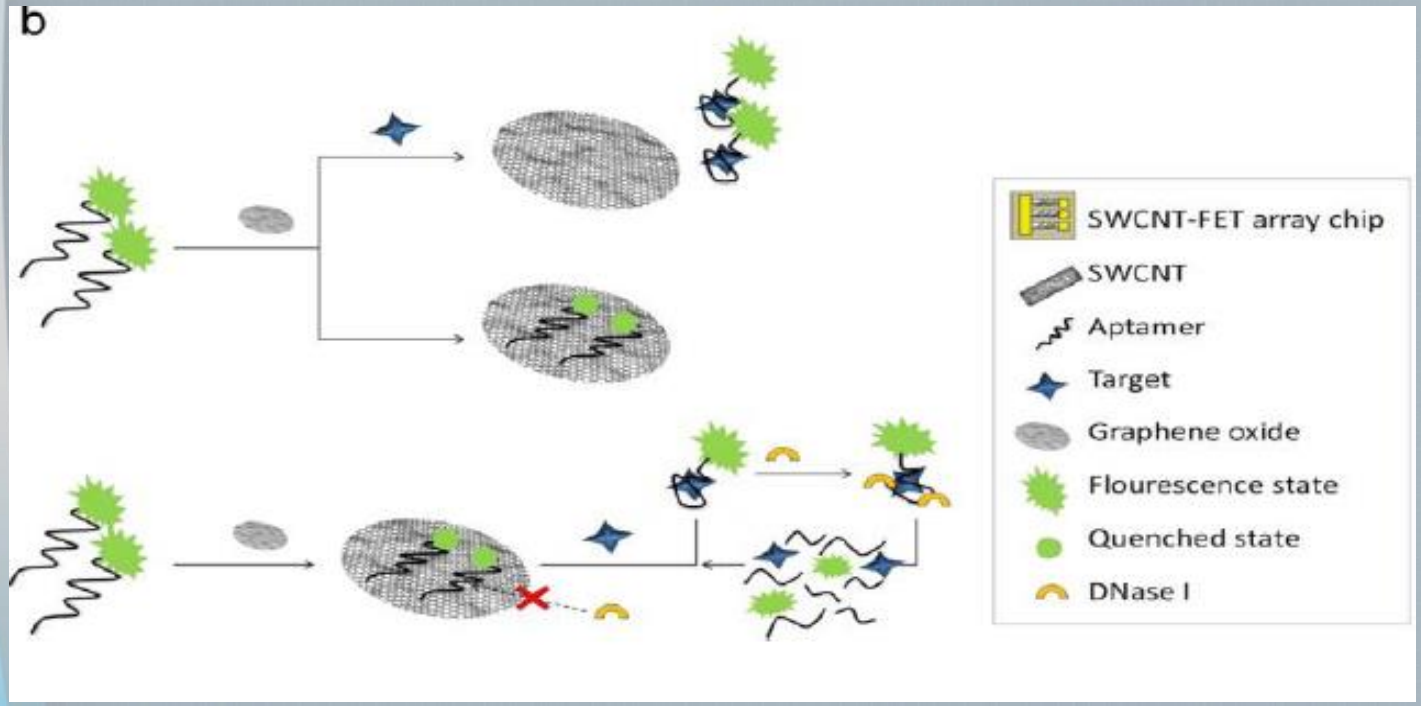
2) mechanical strength

3) thermal and electrical conductivity

Types of transducer and methods

- **Optical Aptasensor ⟶ Nano materials**

single walled carbon nanotubes (SWCNTs) [23]



Optical aptasensors for quantitative detection of small biomolecules: A review

Chunjing Feng, Shuang Dai, Lei Wang*

ABSTRACT

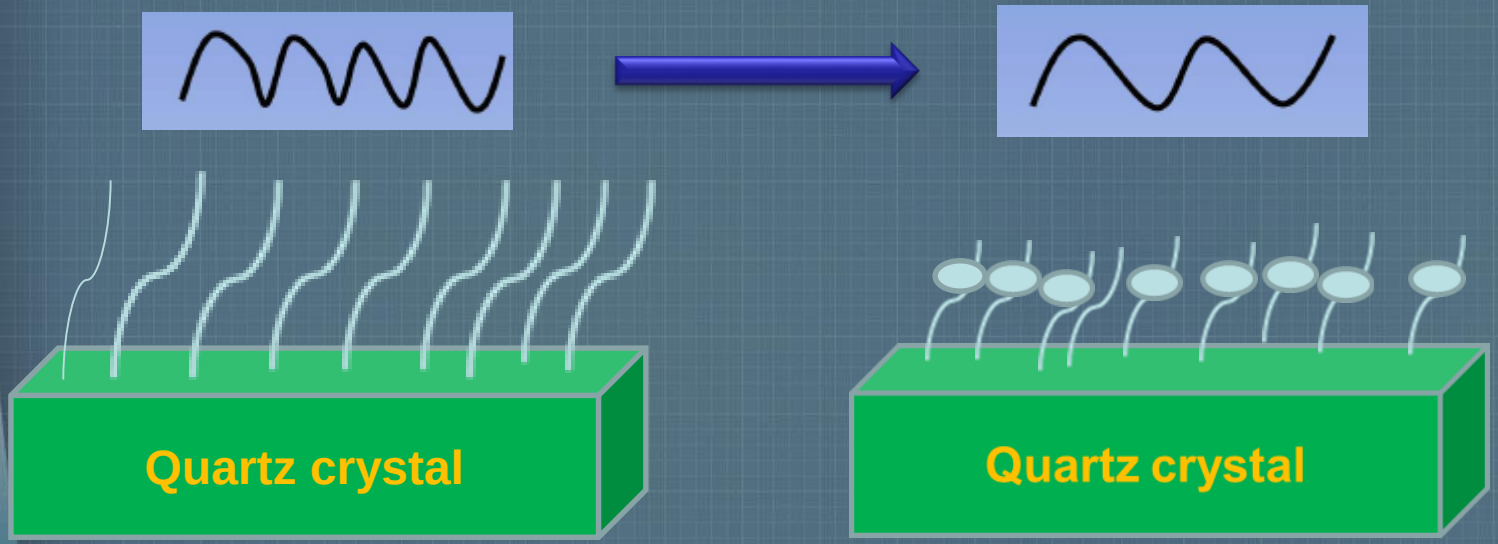
Aptasensors are aptamer-based biosensors with excellent recognition capability towards a wide range of targets. Specially, there have been ever-growing interests in the development of aptasensors for the detection of small molecules. This phenomenon is contributed to two reasons. On one hand, small biomolecules play an important role in living organisms with many kinds of biological function, such as antiarrhythmic effect and vasodilator activity of adenosine. On the other hand, the concentration of small molecules can be an indicator for disease diagnosis, for example, the concentration of ATP is closely associated with cell injury and cell viability. As a potential analysis tool in the construction of aptasensors, optical analysis has attracted much more interest of researchers due to its high sensitivity, quick response and simple operation. Besides, it promises the promotion of aptasensors in performance toward a new level. Review the development of optical aptasensors for small biomolecules will give readers an overall understanding of its progress and provide some theoretical guidelines for its future development. Hence, we give a mini-review on the advance of optical aptasensors for small biomolecules. This review focuses on recent achievements in the design of various optical aptasensors for small biomolecules, containing fluorescence aptasensors, colorimetric aptasensors, chemiluminescence aptasensors and other optical aptasensors.

Types of transducer and methods

- Mass-sensitive

quartz crystal microbalance [4,24]

measuring the change in frequency of a quartz crystal

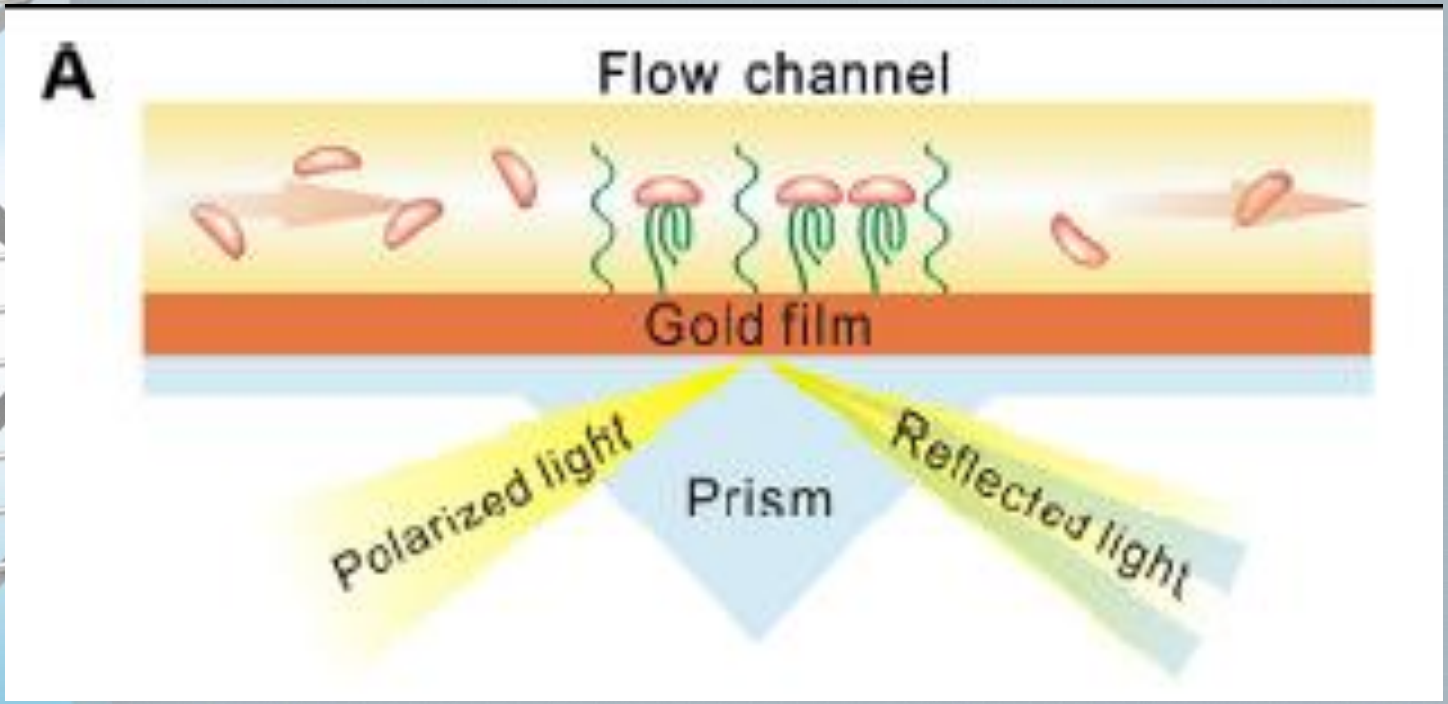


Types of transducer and methods

- **Mass-sensitive** [25]

Surface Plasmon resonance

Vibration of the electron cloud in a molecule



Discussion

Discussion

Aptamer vs antibody

- easier engineering and synthesis [26]
- better thermal stability
- Smaller size
- lower immunogenicity

Discussion

Aptamer vs antibody

	Aptamer	Antibody
Development time	several week	Several months
temperature	Stable	sensitive
Shelf life	year	month
Chemical Modification	easy	limited
Size	small	large

Aptamers: Problems, Solutions and Prospects

ABSTRACT Aptamers are short single-stranded oligonucleotides that are capable of binding various molecules with high affinity and specificity. When the technology of aptamer selection was developed almost a quarter of a century ago, a suggestion was immediately put forward that it might be a revolutionary start into solving many problems associated with diagnostics and the therapy of diseases. However, multiple attempts to use aptamers in practice, although sometimes successful, have been generally much less efficient than had been expected initially. This review is mostly devoted not to the successful use of aptamers but to the problems impeding the widespread application of aptamers in diagnostics and therapy, as well as to approaches that could considerably expand the range of aptamer application.

KEYWORDS SELEX; aptamer; diagnostics; therapeutics; problems.

ABBREVIATIONS NAs – nucleic acids; IOP – initial oligonucleotide pool; PEG – polyethylene glycol; SELEX – systematic evolution of ligands by exponential enrichment; siRNA – small interfering RNA.

Discussion

Aptamer problemes

- ✓ Aptamer degradation
- ✓ Aptamer excretion from the bloodstream by renal filtration
- ✓ Control of the duration of action
- ✓ Generation of aptamers using unpurified target proteins
- ✓ Aptamer cross-reactivity

Discussion

Aptamer degradation

- degradation of aptamers by nucleases in biological media
- unacceptable for most therapeutic applications
- Use oligonucleotides containing modified nucleotides
- Methylation in 2’ c in sugar position
- increase aptamer resistance to nucleases without affecting their binding to target **[27]**

Discussion

Aptamer degradation

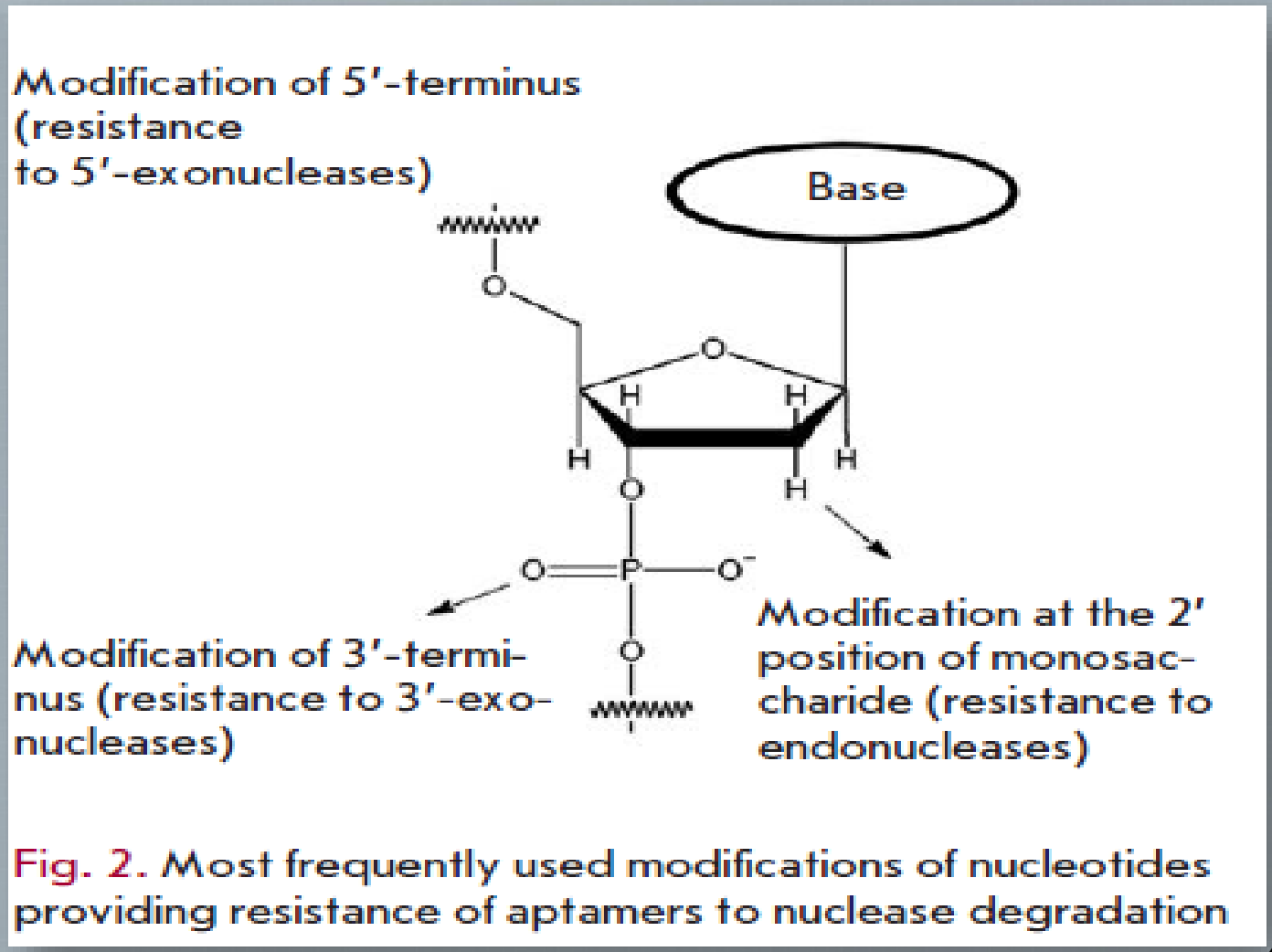


Fig. 2. Most frequently used modifications of nucleotides providing resistance of aptamers to nuclease degradation

Discussion

excretion from the bloodstream

- aptamers weight ranging from 5 to 15 kDa
- kidneys easily removing substances with a molecular weight below 30–50 kDa
- Conjugation of aptamers with polyethylene glycol
- Alternative option also be conjugated with cholesterol

Discussion

Duration of action

- very important in its therapeutic application. [27]

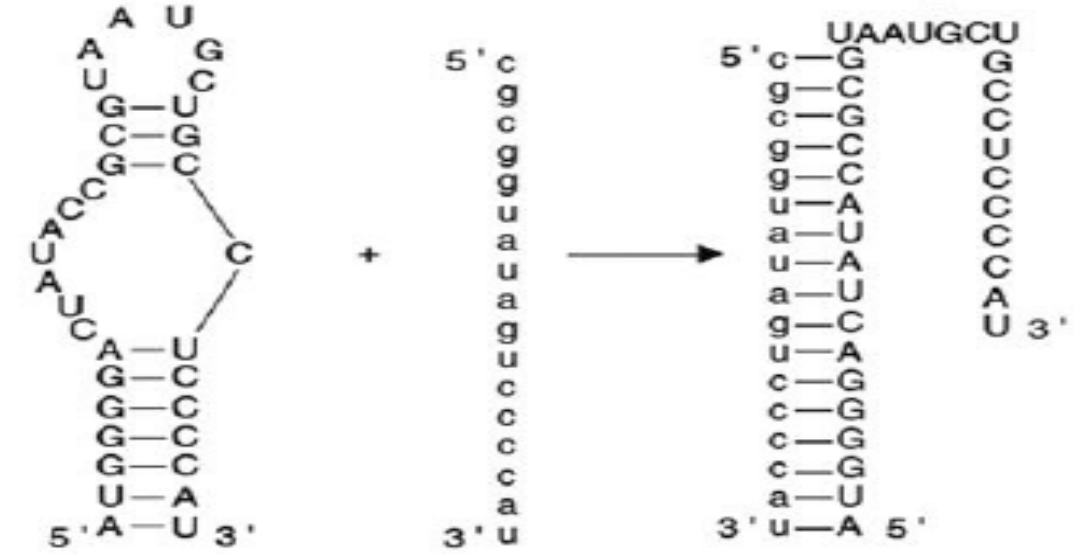


Fig. 5. Antidote-dependent regulation of aptamer functioning. The aptamer 9.3t is shown as an example [77]. This aptamer interacts with the coagulation factor IXa and has anticoagulation properties. Administration of a complementary antidote leads to quick inactivation of this aptamer and restoration of blood coagulation

Discussion

Un purified target proteins

- Aptamer generation requires the availability of purifie molecules.
- Some protein are difficult to purify
- sometimes proteins expressed in prokaryotic cell not good
- These can make epitopes of eukaryotic proteins inaccessible to aptamers
- The modified SELEX protocol (Cell-SELEX) **[27]**

Discussion

Cell selex

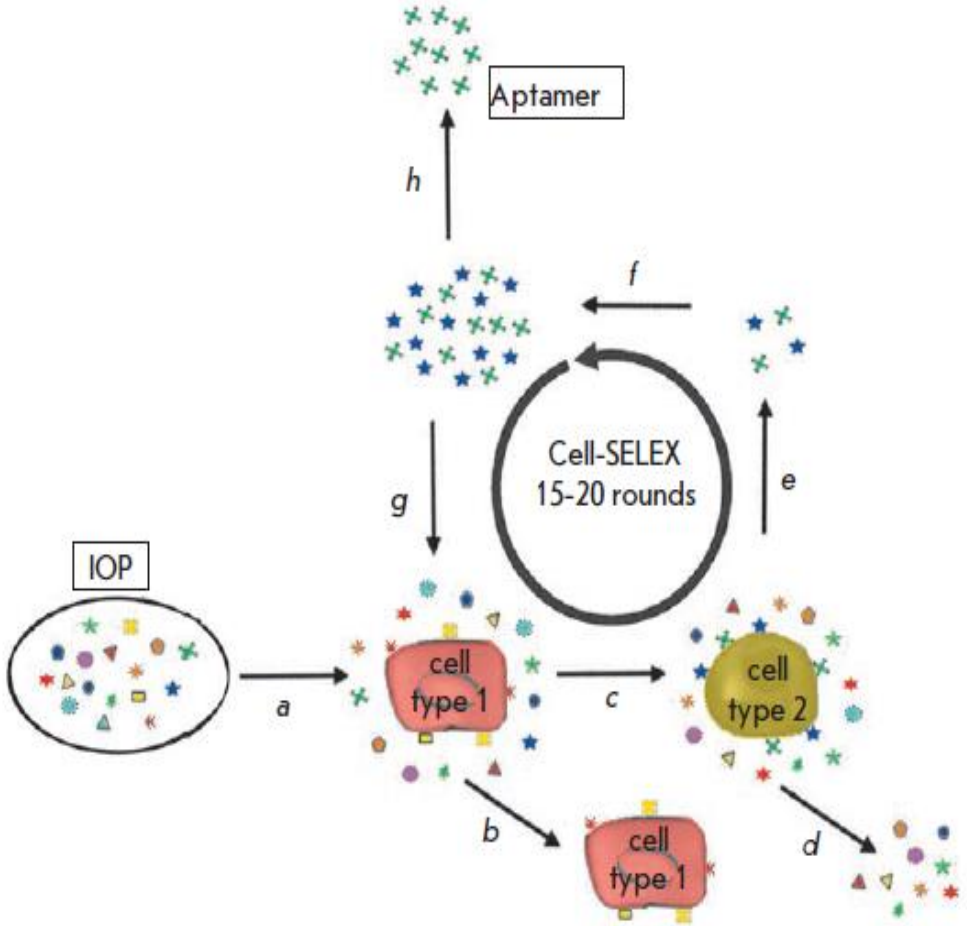
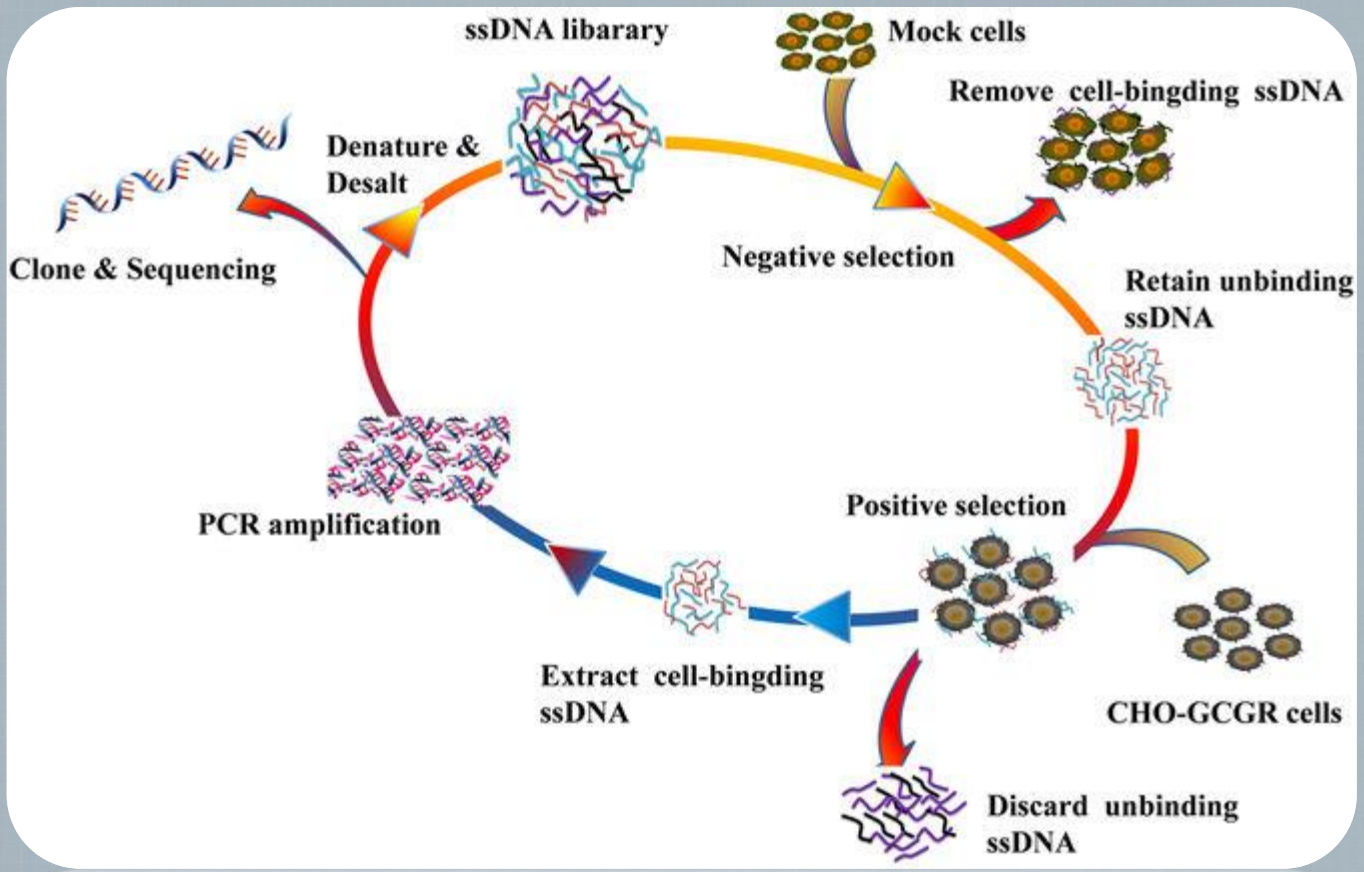


Fig. 6. Scheme of Cell-SELEX. (a) IOP is first incubated with a non-target cell in a negative selection step. (b) All oligonucleotides that show binding to the negative control cells are removed. (c) Unbound oligonucleotides from the negative step are added to the target cells in a positive selection step. (d) Unbound oligonucleotides from the positive step are separated from bound molecules by washing steps. (e) Oligonucleotides binding target cells are subsequently eluted. (f) Eluted oligonucleotides are amplified using the PCR (DNA-SELEX) or RT-PCR (RNA-SELEX) technique. (g) The enriched pool is then subjected to further rounds of selection. (h) After 15–20 rounds, aptamers are cloned and analyzed in detail

Discussion

Aptamer cross-reactivity

- aptamers that recognize with a similar structure. DNA polyβ, κ



Conclusion

Conclusion

conclusion

- **Aptasensors emerged only about 10 years ago**
- **basic research and biomedical diagnostics**
- **SELEX is a procedure necessary for development of aptamers for certain ligand**
- **Few days for aptamer ,several month for antibody**

Conclusion

conclusion

- **Limitation the use of Aptamers in treatment**

  Macugen ⟶ VEGF

- **The use of aptamers in diagnostics has fewer limitations**

- **Limitation on diagnosis: lack of standardized protocols**

- **Solution:**

generating standardized kits and protocols based on well-characterized aptamers with optimum characteristics [27]

references

1) Radi A-E. Electrochemical aptamer-based biosensors: recent advances and perspectives. International Journal of Electrochemistry. 2011;2011.
2) Ling ZH, Ming-Hua WA, Jian-Ping WA, Zhun-Zhong YE. Application of biosensor surface immobilization methods for aptamer. Chinese Journal of Analytical Chemistry. 2011 Mar 1;39(3):432-8.
3) O'Sullivan CK. Aptasensors-the future of biosensing?. Analytical and bioanalytical chemistry. 2002 Jan 1;372(1):44-8.
4) Song S, Wang L, Li J, Fan C, Zhao J. Aptamer-based biosensors. TrAC Trends in Analytical Chemistry. 2008 Feb 29;27(2):108-17.
5) Nimse SB, Song K, Sonawane MD, Sayyed DR, Kim T. Immobilization techniques for microarray: challenges and applications. Sensors. 2014 Nov 25;14(12):22208-29.
6) Pan S, Rothberg L. Chemical control of electrode functionalization for detection of DNA hybridization by electrochemical impedance spectroscopy. Langmuir. 2005 Feb 1;21(3):1022-7.
7) Fixe, F.; Dufva, M.; Telleman, P.; Christensen, C.B.V. Functionalization of poly(methyl methacrylate) (PMMA) as a substrate for DNA microarrays. Nucleic Acids Res. 2004, 32, doi:10.1093/nar/gng157.
8) Chrisey, L.A.; Lee, G.U.; O'Ferrall, C.E. Covalent Attachment of Synthetic DNA to Self-Assembled Monolayer Film. Nucleic Acids Res. 1996, 24, 3031–3039.

references

9) Gajovic-Eichelmann, N.; Ehrentreich-Forster, E.; Bier, F.F. Directed immobilization of nucleic acids at ultramicroelectrodes using a novel electro-deposited polymer. Biosens. Bioelectron. 2003, 19, 417–422.
10) Rogers, Y.H.; Jiang-Baucom, P.; Huang, Z.J.; Bogdanov, V.; Anderson, S.; Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. Anal. Biochem. 1999, 266, 23–30.
11) Hayat A, Marty JL. Aptamer based electrochemical sensors for emerging environmental pollutants. Frontiers in chemistry. 2014;2.
12) B. Y. Kim, C. B. Swearingen, J. A. Ho, E. V.Romanova, P. W. Bohn and J. V. Sweedler,J. Am. Chem. Soc., 2007, 129, 7620–7626.
13) Ling ZH, Ming-Hua WA, Jian-Ping WA, Zhun-Zhong YE. Application of biosensor surface immobilization methods for aptamer. Chinese Journal of Analytical Chemistry. 2011 Mar 1;39(3):432-8.
14) Zhao W, Chiuman W, Brook MA, Li Y. Simple and rapid colorimetric biosensors based on DNA aptamer and noncrosslinking gold nanoparticle aggregation. ChemBioChem. 2007;8(7):727-31.
15) Feng C, Dai S, Wang L. Optical aptasensors for quantitative detection of small biomolecules: A review. Biosensors and Bioelectronics. 2014 Sep 15;59:64-74.

references

16) Chang H, Tang L, Wang Y, Jiang J, Li J. Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection Analytical Chemistry.2010;82(6):2341-6.

17) E Wang R, Zhang Y, Cai J, Cai W, Gao T. Aptamer-based fluorescent biosensors. Current medicinal chemistry. 2011 Sep 1;18(27):4175-84.

18) Kim S-E, Ahn K-Y, Park J-S, Kim KR, Lee KE, Han S-S, et al. Fluorescent ferritin nanoparticles and application to the aptamer sensor. Analytical chemistry. 2011;83(15):5834-43.

19) Burda C, Chen X, Narayanan R, El-Sayed MA. Chemistry and properties of nanocrystals of different shapes. Chemical reviews. 2005;105(4):1025-102

20) Feng C, Dai S, Wang L. Optical aptasensors for quantitative detection of small biomolecules: A review. Biosensors and Bioelectronics. 2014 Sep 15;59:64-74.

21) Lu D, He L, Zhang G, Lv A, Wang R, Zhang X, et al. Aptamer-assembled nanomaterials for fluorescent sensing and imaging. Nanophotonics. 2017;6(1):109-21.

22) So H-M, Won K, Kim YH, Kim B-K, Ryu BH, Na PS, et al. Single-walled carbon nanotube biosensors using aptamers as molecular recognition elements. Journal of the American Chemical Society. 2005;127(34):11906-7

23) Chen D, Feng H, Li J. Graphene oxide: preparation, functionalization, and electrochemical applications. Chemical reviews. 2012;112(11):6027-53.

references

24) Lim YC, Kouzani AZ, Duan W. Aptasensors: a review. Journal of biomedical nanotechnology. 2010 Apr 1;6(2):93-105.

25) Vo-Dinh T, Cullum B. Biosensors and biochips: advances in biological and medical diagnostics. Fresenius' journal of analytical chemistry. 2000 Mar 1;366(6):540-51.

26) Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nature reviews Drug discovery. 2010;9(7):537-50

27) Lakhin AV, Tarantul VZ, Gening LV. Aptamers: problems, solutions and prospects. Acta Naturae (англоязычная версия). 2013;5(4 (19)).